Effects of Seed Maturation Temperature on Seed Yield Characteristics and Subsequent Generations of Lupin

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ABSTRACT

Phenology and morphology of white lupin (*Lupinus albus* L.) varied in plants derived from different seed lots of the same cultivar, presumably because thermosensitive genotypes are affected by thermal environment during early development. This study was conducted to determine whether the maternal thermal environment of developing seeds influenced subsequent plant development. We grew white lupin plants cv. Ultra, at the mainstem first flower stage, in controlled growth chambers at 13 or 28C temperature under a l4-h photoperiod. At harvest maturity, yield components were quantified. The two resultant seed populations were planted in the greenhouse, and at the mainstem first flower stage, one-half of each group of plants was placed in growth chambers at 13 or 28°C. This regimen was carried out through three generations. Seed dry weight per plant and weight per seed were greater for plants matured at 13°C than at 28°C. All plants produced from maternal seed that had matured at 28°C developed at a slower rate than those at 13°C. Floral events in these plants lagged behind by as much as 15 d. No differences were observed in mainstem vegetative node number. We concluded that phenotypic variability among seedlots of the same genotype can be caused by thermal environmental conditions occurring as embryos develop. These results show that thermal environment during seed production can affect subsequent crop performance, which can impact cultivar production and consistency among years.

T EMPERATURE during early seedling development influences growth, development, and time of harvest of white lupin (Clapham and Willcott, 1995; Rahman and Gladstones, 1972; Williams and Brocklehurst, 1983). Cold temperatures during early seedling development cause a reduction in the number of vegetative nodes (Putnam et al., 1993; Williams and Brocklehurst, 1983) and earlier floral initiation (Bradley, 1982; Rahman and Gladstones, 1972). The term *vernalization* is commonly used in the literature to describe cold temperature effects on germinating seeds. Vernalization is not required for spring morphotypes of white lupin to complete their life cycle, but can have a profound impact on their phenology and morphology, as well as reduce seed yield (Bradley, 1982; Putnam et al., 1993; Williams and Brocklehurst, 1983).

Clapham and Willcott (1995) observed that spring white lupin is responsive to warm temperatures as well as cold temperature ranges during germination. The multiple physiological responses to warm and cold temperatures suggest that the term *thermosensitivity* may be more appropriate than vernalization when describing morphological and phenological responses to temperature. Temperature sensitivity during early development

is associated with variability in time of harvest and is probably one of the factors that limit use of spring white lupin as a grain legume in regions with cool, short growing seasons (G. Gataulina, 1996, personal communication).

In a series of growth chamber experiments, we observed that various seedlots of Ultra white lupin produced plants that differed significantly in development and morphology under similar conditions. Because most spring morphotypes of white lupin are thermosensitive, we hypothesized that the thermal environment in which the various seed lots were produced could have had an influence on embryo development, which would be expressed during subsequent seedling development. Thermal environment during embryo development has an important influence on the subsequent generation in other plants. Gregory and Purvis (1938) demonstrated that developing embryos of winter rye (*Secale cereale* L.) could be vernalized. Vernalization of immature embryos of winter wheat (*Triticum aestivum* L.) was related to age and endogenous phytohormones (Dubert et al., 1993). Vernalization of parents of pea (*Pisum sativum* L.) during embryo development was shown to affect the flowering node of the progeny (Reid, 1979). Riddell and Gries (1958) observed in winter wheat that plants grown from seed matured at 16°C had fewer nodes and flowered earlier following vernalization than did plants grown from seed matured at 27°C.

We hypothesized that seed matured in a cool temperature environment would produce fewer embryonic leaves than seed matured at warmer temperatures. These morphological differences might be expressed in the vegetative development of the subsequent generation. Development of new crops requires predictable cultivar performance, and sources of variability must be documented and understood. The objectives of this study were to determine the effects of temperature during seed maturation on the seed yield of treated plants and the subsequent phenological development of daughter plants.

MATERIALS AND METHODS

Propagation Conditions

Ultra white lupin was propagated under controlled environmental conditions through three generations to determine the effects of seed maturation environment on seed yield parameters and subsequent development. Prior to planting, seeds were surface sterilized for 2 min with a 0.066 *M* NaHOCl solution and then rinsed with deionized water. Fungal pathogens were controlled by imbibing seeds in a jar of deionized water containing 2 mg L^{-1} metalaxyl [(*N*-2, 6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester)] for 24 h at 18°C. Imbibed seeds were sown in Jiffy-7 peat plugs (Jiffy Products,

Table 1. Phenological Index for developmental events from seedling to first-order lateral flowering stages.

Index	State of development
	Vegetative growth stages
1	Mainstem leaf 1 fully expanded (first leaf above cotyledons)
2	Leaf 2
3	Leaf 3
16	Leaf 16 fully expanded
	Mainstem reproductive stages
18	Mainstem first flower. First flower open on mainstem raceme
19	Mainstem full flower. Most flowers open on mainstem raceme at the same time. First flowers are senescing.
20	Mainstem last flower. Distal flower on mainstem raceme is open, proximal flowers senescent and/or proximal mainstem raceme nodes set pods.
	First-order reproductive stages
22	First-order lateral first flower. First flower open on first-order branch raceme(s)
23	First-order lateral full flower. Most flowers open on first-order raceme(s) open at the same time.
24	First-order lateral last flower. Distal flower on first-order raceme(s) open, proximal flowers senescent, proximal raceme nodes set pods.

Shippagan, Canada) and planted into 23-cm-diam. pots (6 plants pot^{-1}) in a peat–vermiculite–perlite planting medium.[1]

Three seed yield experiments were conducted (Exp. 1, 2, and 3). A commercial source (Seed Lot 108, L&T Enterprises, Perham, MN) was used as the maternal seed (the seed that was planted) in Exp. 1. The seeded pots were placed in a greenhouse maintained at a 14-h day length with 18°C days and 13°C nights until mainstem full flower (Stage 21, Table 1). (All greenhouse phases were conducted during winter months (1994–1996) when light and temperature conditions could be controlled). Plants were then moved to constant-temperature, controlled-£environment growth chambers at either 13 or 28°C

(14-h day length). Plants self-pollinated and set seed in the constant-temperature environment where they remained until pods had desiccated and seed had matured. Light was provided by a combination of high pressure Na and metal halide lamps (700 μmol photon m^{-2} s^{-1}). Plants were fertilized weekly with 500 mL of one-half strength Hoagland's solution (Hoagland and Arnon, 1950). Seeds produced by the mainstem and first-order lateral branches were counted and weighed at harvest maturity. All harvested seed (herein referred to as daughter seed) was stored at room temperature for <30 d prior to replanting in subsequent experiments.

A portion of the daughter seed from each treatment in Exp. 1 was used as the maternal seed for Exp. 2. Again the pots were maintained in the greenhouse until full flower and moved into constant temperature growth chambers at either 13 or 28°C (14-h day length) until seed set and maturation. There were four treatments in this experiment, representing the two maternal and two daughter seed-maturation temperatures designated 13/13, 13/28, 28/13, and 28/28 (treatment nomenclature follows Gorecki et al., 1996). The four sets of daughter seed produced in Exp. 2 were counted and weighed at maturity.

Daughter seeds from Exp. 2 were then pooled based on their maturation temperature (i.e., seeds from treatment 13/13 and 28/13 were combined into one seed group representing seed matured at 13°C and seeds from treatments 13/28 and 28/28 were combined in a separate group of seed matured at 28°C). (Pooling was necessary to produce sufficient number of seeds for replanting). A portion of each group of daughter seeds from Exp. 2 was then used as the maternal seed for Exp. 3. Experiment 3 thus repeated Exp. 2 in that the effects of the same four treatments representing the two maternal and two daughter maturation temperatures were examined. Again seed parameters were measured at maturity.

Phenology

A second series of experiments was conducted to determine the effect of maternal seed maturation temperature on daugh-

[1] Mention of brand name or trademark does not constitute endorsement of the product by USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Table 2. Seed yield components from three generations of white lupin matured at different temperatures.

		Seed maturation temperature		Seed number		Seed yield		Seed dry wt	
Experiment	Source of variation	Maternal generation	Daughter generation	Mainstem	First-order lateral branches	Mainstem	First-order lateral branches	Mainstem	First-order lateral branches
		——°C——		—— seeds plant^{-1} ——		—— g plant^{-1} ——		—— g seed^{-1} ——	
1		–†	13	4	2	0.71	0.39	0.14	0.14
		–	28	6	0	0.25	0	0.04	0
	Daughter generation temperature			**	***	***	***	***	***
2		13	13	9	9	2.25	2.1	0.26	0.24
		13	28	7	2	0.61	0.16	0.08	0.06
		28	13	9	6	2.82	1.5	0.3	0.24
		28	28	11	4	0.88	0.38	0.08	0.08
	Maternal generation temperature			***	NS	**	NS	NS	NS
	Daughter generation temperature			NS	***	***	***	***	***
	Maternal × daughter			**	**	NS	*	*	NS
3		13	13	8	15	2.51	4.22	0.29	0.28
		13	28	5	4	1.69	1.03	0.34	0.28
		28	13	10	19	3.23	5.94	0.32	0.30
		28	28	6	4	2.03	1.22	0.33	0.30
	Maternal generation temperature			NS	NS	NS	NS	NS	NS
	Daughter generation temperature			**	**	**	**	NS	NS
	Maternal × daughter			NS	NS	NS	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 levels of probability, respectively; NS is not significant.
† Commercial seed source with unknown maturation temperature.

ter plant phenology. Portions of each group of the daughter seed produced from Exp. 1, 2, and 3 were used as maternal seeds in Exp. 1P, 2P, and 3P, respectively. (Effects of both the maternal and grandmaternal seed maturation temperature were evaluated in Exp. 2P and 3P.) Seeds, which had been stored at room temperature for <30 d after harvest, were rinsed, imbibed, and planted as described above. The plants were maintained under the greenhouse conditions described above until completion of first-order, lateral-branch flowering. A phenological index (Table 1) was created to describe the developmental stages of spring white lupin. Plants were monitored daily and the date was recorded when each developmental stage was attained.

Analysis

Each experiment was a completely randomized design with six replicates. Data collected were analyzed using the Statistics and MGLH modules of Systat (Systat, Evanston, IL) and StatView (SAS Institute, Cary, NC). Separate analyses of variance were performed on the seed yield data from Exp. 1, 2, and 3 using the appropriate error mean square variances (based upon expectations of mean squares) (Steel and Torrie, 1960). Significance of differences in plant development due to maternal seed environment (Exp. 1P, 2P, and 3P) was determined by T test for the dates that specific growth stages were achieved.

RESULTS

Seed Characteristics

Seed maturation temperature had significant effects on all measured seed parameters (Table 2). In the following discussion plants that were moved from the greenhouse to a 13°C growth chamber at mainstem full flower, where they remained until all mainstem and first-order branch seed were mature and dry, are described as plants matured at 13°C. Plants treated similarly in a 28°C growth chamber are described as plants matured at 28°C.

Experiment 1

Plants matured at 13°C produced 25% fewer mainstem seeds than plants matured at 28°C (Table 2). First-order branches supported more than one-third of the total seeds produced at 13°C. Plants matured at 28°C failed to produce any first-order lateral branch seeds. Seeds matured at 13°C were three times larger (g seed^{-1}) than those matured at 28°C, and total seed yield (g plant^{-1}) at 13°C was nearly three times greater than at 28°C.

Experiments 2 and 3

The effects of both the maternal and daughter seed maturation temperatures were assessed in Exp. 2 and 3 (Table 2). Effects related to the temperatures experienced by the daughter generation were similar to the results of Exp. 1. Plants matured at 13°C had greater production from first-order branches and consistently greater yield per plant and weight per seed. Maternal seed maturation temperature had significant effects on some seed parameters in Exp. 2. Both main stem seed
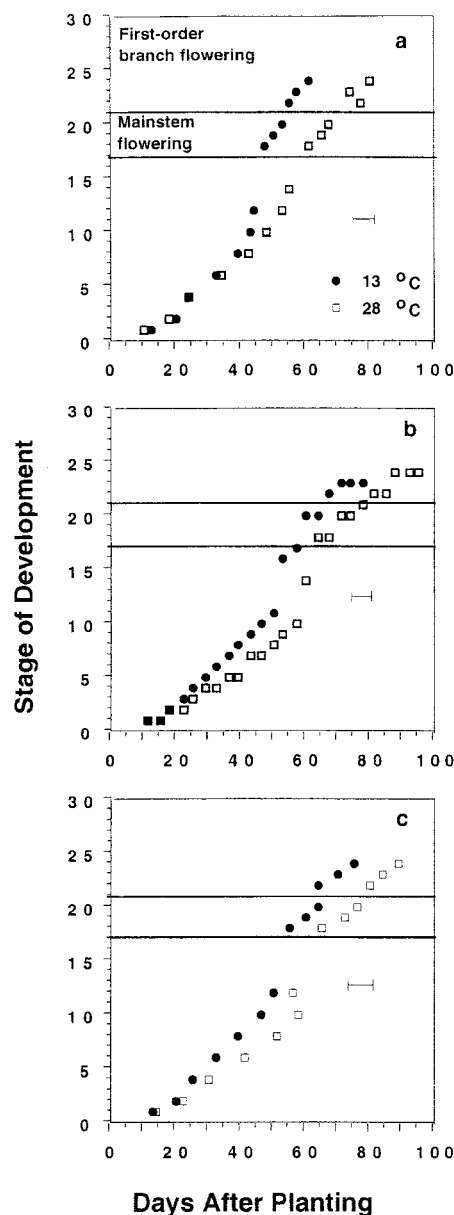

Fig. 1. Phenology of three generations of lupin seedlings derived from seeds matured at 13 or 28°C: (a) Exp. 1P, (b) Exp. 2P, (c) Exp. 3P. Symbols represent the mean date that a growth stage was first achieved. Error bars are two times the pooled standard error of the mean for each experiment.

number and seed yield were more than 25% higher for plants with a maternal seed maturation temperature of 28°C. Similar but nonsignificant trends were observed in Exp. 3.

Phenology

The developmental rates of plants were affected by the maturation temperature of the maternal seed. (The maturation temperature of the grandmaternal seed had no significant effect on plant development rate.) Data in Fig. 1 show the mean development patterns for plants whose maternal seeds were matured at either 13 or 28°C. Plants produced from seed matured at 13°C were generally healthier and grew more vigorously than

plants from seed matured at 28°C. Treatments did not affect mainstem vegetative node number at maturity ($P < 0.001$). Early development of plants from seed matured at 13 or 28°C was similar up to Stage 6 (sixth mainstem vegetative node) in Exp. 1P (Fig. 1a). After Stage 6, the plants from 28°C seed developed slower than plants from 13°C seed. For example, plants in Exp. 1P that grew from seed matured at 13°C reached Stage 18 (mainstem first flower) on Day 47 and Stage 24 (first-order lateral branch last flower) on Day 61, while plants from 28°C seeds reached Stage 18 on Day 61 and Stage 24 on Day 80. A similar pattern was observed in Exp. 2P (Fig. 1b), with the lag first observed at Stage 3 (third vegetative node). Seed maturation temperature effects on floral events in Exp. 2P were not as great as in Exp. 1P, with lags of ~10 d. In Exp. 3P (Fig. 1c), the lag in seedling development was first observed at Stage 3. Mainstem first flower occurred 10 d later in the 28°C than in the 13°C derived seedlings, and Stage 24 was reached 15 d later in the 28°C seedlings.

DISCUSSION

Temperature during maturation of the daughter seed affected seed yield parameters. Specifically, the cooler maturation temperature of the daughter generation resulted in greater mass per seed, seeds per plant, and total seed yield (g plant^{-1}). These data are consistent with observations made for soybean [*Glycine max* (L.) Merrill] (Egli and Wardlaw, 1980), where seed size reductions were attributed to a reduction in seed growth rate due to accelerated leaf senescence at higher temperatures. Leaf senescence was also accelerated in the 28 vs. 13°C environment in these studies (data not shown). An additional consequence of accelerated leaf senescence in the 28°C environment was a reduction in the duration of pod filling (data not shown). The reduction in the duration of pod filling was consistent with observations made in common bean (*Phaseolus vulgaris* L.) (Scully and Wallace, 1990).

Temperature during maturation of the maternal seed affected phenology and, in some cases, seed yield parameters of the daughter generation. Plants growing from maternal seed that had matured at 28°C temperatures developed more slowly, reaching Growth Stage 24 from 10 to 19 d later than seed matured at lower temperatures (Fig. 1). This difference in developmental rate could reduce crop yields in situations where field establishment is delayed or the growing season is short. Interestingly, seed yield could be higher from daughter plants whose maternal seed was matured at the higher temperature (Table 2). Slower development may increase duration of light capture and thus photosynthate available for seed production.

Variation in agronomic characters as the result of adverse environments was observed in flax (*Linum angustifolium* Huds.) and was attributed to instability of some genes associated with reductions in the amount of DNA (Durrant, 1981). Shatters et al. (1995) examined polymorphisms in four populations of soybean seeds, which were produced by treating a single genotype with

a range of temperature, humidity conditions, and controlled hydration (seed priming). Our results differed from Highkin (1958) who found a carryover of the effects of maternal seed maturation temperature through up to five generations of pea. Our observations are consistent with the empirical explanation offered by Scully and Wallace (1990) and Egli and Wardlaw (1980) that effects of warmer temperatures during embryo development were manifest in shorter pod fill duration and earlier leaf senescence that resulted in lower seed yields and vitality.

The compelling reason to examine the role of maternal environment on subsequent generation lupin development was driven by greenhouse and field observations of differences in phenology and morphology among different seed lots of the same genotype. Spring morphotypes of white lupin vary in their response to temperature early in their development (Clapham and Willcott, 1995), possibly due to maternal temperature effects on embryo and leaf primordia development in ways similar to spring wheat (Riddell and Gries, 1958), winter wheat (Weibel, 1958), and winter rye (Gregory and Purvis, 1938). We hypothesized that seed matured in a cool temperature environment (13°C) would produce fewer embryonic leaves than seeds matured at 28°C. These morphological differences might be expressed in the vegetative development of the subsequent generation. Data collected in these studies do not support this hypothesis. Therefore, differences in phenology in lupin plants resulting from seeds matured at 13 and 28°C are probably due to the physiological state of the seed, seed size, or to some other antecedent factor.

The temperature conditions during seed development and maturation could account for variation among different seedlots of the same genotype. This study indicates that environmental conditions during seed production can be important in determining the performance of the progeny. Our data suggest that consistency of maturation environment across years and sites could be important for producing a more consistent crop performance.

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SEED PHYSIOLOGY, PRODUCTION & TECHNOLOGY

White Clover Seed Production: III. Cultivar Differences under Contrasting Management Practices

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ABSTRACT

Information is needed to determine the optimal combinations of agronomic practices for white clover (*Trifolium repens* L.) seed production in humid temperate marine climatic conditions. Effects on seed yield were determined for two stand ages (first and second seed year of production), six cultivars varying in leaf size (ladino large-leaf type: Canopy, California Ladino, Osceola, and Regal; and intermediate leaf-type: Louisiana S-1 and White Dutch), grown with and without spring herbage removal, and with and without supplemental irrigation. The experiment was arranged in a modified split-split-split-plot design with four replications. First year seed was harvested in 1997, and first and second year seed was harvested in 1998. Intermediate-leaf size cultivars reached initial bud and flower stages earlier than large-leaf types, but there were no differences between the two leaf types at the time of seed harvest. The number of flowers produced early in the reproductive period for nonirrigated plants was highly correlated with seed yield. Supplemental irrigation delayed flower maturity, but herbage removal did not. First seed year yields were greater than second seed year yields for all cultivars except Osceola, which had similar yields both seed years. Supplemental irrigation only increased seed yields of Canopy. In first seed year stands, White Dutch was the only cultivar that did not recover lost seed yields due to herbage removal when supplemental irrigation was applied. Herbage removal and supplemental irrigation treatments generally did not increase white clover seed yields under the conditions of this experiment, so maximal yields were generally achieved with minimal management inputs.

PACIFIC NORTHWEST GRASS SEED cropping systems have historically relied on disturbance practices, large quantities of purchased agricultural chemicals, and open-field burning to establish new crops, control diseases and weeds, and dispose of postharvest residues to achieve high seed yields with good seed quality. Many of these historic systems have been continuous grass seed monocultures, and thus have lacked rotation crop diversity. In western Oregon, white clover grown for seed is one of several minor crops that can provide rotation crop options for grass seed production systems on poorly drained soils. For the production of economic amounts of good quality seed, alternative conservation systems are needed to manage forage and turfgrass seed crops in the absence of open-field burning and with reduced chemical inputs.

White clover is a broadly adapted perennial forage legume that grows under a wide range of climatic conditions. Many cultivars are grown throughout the United States for herbage, but seed production is limited to regions within the western states (Steiner, 1994). The climatic conditions in western Oregon are similar to those found in western Europe and New Zealand, but are very different from the arid Mediterranean-like conditions found in the irrigated Great Central Valley of California (Bailey, 1996) where white clover seed is also produced. Approximately 1200 ha of seed are produced in California (J. Reich, 1998, personal communication) and 600 ha in western Oregon (E. Edminster, 1998, personal communication). Historic production in California and Oregon were ≈13 000 and 10 000 ha, respectively (Scullen, 1952). The ladino large-leaf type cultivars California Ladino, Canopy, Osceola, and Regal are typically grown for seed in California, but can also be grown in Oregon. The intermediate leaf-type cultivars Louisiana S-1 and White Dutch are typically grown in

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